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Calcium channel functions in pain processing

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Voltage-gated calcium channels (VGCC) play obligatory physiological roles, including modulation of neuronal functions, synaptic plasticity, neurotransmitter release and gene transcription. Dysregulation and maladaptive changes in VGCC expression and activities may occur in the sensory pathway under various pathological conditions that could contribute to the development of pain. In this review, we summarized the most recent findings on the regulation of VGCC expression and physiological functions in the sensory pathway, and in dysregulation and maladaptive changes of VGCC under pain-inducing conditions. The implications of these changes in understanding the mechanisms of pain transduction and in new drug design are also discussed.

Introduction

Pain information processing starts from activation of peripheral nociceptors, causing action potentials to propagate along the primary afferent nerve fibers into sensory neurons in dorsal root ganglia (DRG). They are further relayed to the spinal dorsal horn through the central axons of sensory neurons. The action potentials reaching the central terminals of sensory afferents can cause membrane depolarization, activation of voltage-gated calcium channels (VGCCs), and calcium influx, which triggers synaptic vesicle exocytosis. This then leads to the release of excitatory neurotransmitters including glutamate, pain-inducing peptides such as substance P and calcitonin gene-related peptide (CGRP) into the synaptic cleft. These neurotransmitters can then cause activation of post-synaptic dorsal horn projection neurons and interneurons, leading to spinal modulation of sensory signals. Certain types of VGCC can also regulate the excitability of DRG primary sensory neurons and dorsal horn neurons. In addition, VGCCs may contribute to ascending and descending modulation of sensory signals. Thus, changes in expression and functions of VGCCs in pain-inducing conditions can be potential targets for chronic pain management.

VGCCs can be classified based on their voltage activation characteristics as high- or low-voltage activated channels. The VGCCs can be further subdivided based on their structural similarities of the channel-forming α_1-subunit (Ca_V1, Ca_V2, Ca_V3) or their sensitivity to blockade by pharmacological agents (L, N, P/Q, R and T-type). Collectively, the high-VGCCs include L-($Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$, $Ca_V1.4$), P/Q-($Ca_V2.1$), N-($Ca_V2.2$) and R-($Ca_V2.3$) type channels, while the low-VGCCs include T-type ($Ca_V3.1$, $Ca_V3.2$, $Ca_V3.3$) channels. The high-VGCCs typically form heteromultimers that consist of the channel-forming α_1-subunit along with auxiliary β-, $\alpha_2\delta$ and γ-subunits.[1] Even through conclusive findings are not yet available, data from co-expression and electrophysiological recording experiments support that the low-VGCCs seem to be α_1-subunit monomers.[2]

So far, ten α_1-subunits have been identified in mammals and are encoded by distinct genes.[1] This subunit is also subjected to alternative splicing.[3-11] The α_1-subunit consists of four homologous domains (I–IV), each having six transmembrane helices (S1 through S6), which together form the calcium conduction pore, voltage sensors and gating apparatus.[12] The S4 transmembrane domain contains positive charged amino acids for voltage sensing. There are four known β-subunits (β-1 through β-4), which are intracellular proteins that enhance cell surface expression of the α_1-subunits and modulate the gating properties through their interactions with the channel-forming α_1-subunit and intracellular signaling molecules.[13-15] Four $\alpha_2\delta$-subunits have been identified ($\alpha_2\delta$-1 through $\alpha_2\delta$-4), each consisting of two disulfide-linked peptides (α_2 and δ) that are encoded by the same gene.[16,17] Similar to the β-subunit, $\alpha_2\delta$ subunits promote and stabilize cell surface expression of VGCCs.[18,19] Eight γ-subunits have been identified and appear to act as glycoproteins with four transmembrane segments, but the exact function of the γ-subunit is not well defined.[20,21] Together, the auxiliary subunits modulate the functional properties of the α_1-subunit.

The biophysical properties of the channel-forming α_1-subunit and tissue-specific distribution of VGCCs play a critical role in governing different pathophysiological functions of VGCCs.[1] Regulation of VGCC function by various signaling molecules and pathways adds another level of control. In addition, alternative splicing could control the coupling of VGCCs to signaling pathways and ultimately their functions.[3,6,8,11,22-27] For example, Ca_V1 VGCCs and Ca_V2 VGCCs are regulated by protein phosphorylation and G-proteins, respectively.[28] Ca_V3 VGCCs are regulated by various kinases and G-protein pathways as summarized by recent reviews in references 21 and 29. Based on the functional diversity, tissue specific distribution and coupling to different signaling pathways, these VGCCs play distinct roles in physiological processing of sensory information. In addition, they could become the target of maladaptive neuroplasticity under pain-inducing conditions, leading to the development of sensory hyperexcitability and behavioral hypersensitivity (pain).

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Therefore, correcting VGCC dysregulation and maladaptive changes in the sensory pathway represents an attractive approach for the development of therapeutic agents for tailored pain management under different pain-inducing etiologies. Accordingly, the focus of this review is on our recent understanding of VGCC regulation and function in the sensory pathway under normal and pain-inducing conditions and the potential contributions of changes in VGCC expression/function to pain processing.

L-Type Voltage-Gated Calcium Channels

L-type channels are widely distributed in the central nervous system, cardiac muscles, smooth and skeletal muscles, retina, sinoatrial node and cochlear hair cells.[20,30] In the superficial dorsal horn, L-type channels are expressed mainly on neuronal cell body and dendrites, mediating the activation of calcium-dependent enzyme activities, gene transcription, synaptic signaling and plasticity, as well as the activation of other ion channels such as calcium-activated potassium channels.[31-35] Membrane depolarization with high voltages results in prolonged activation of L-type channels due to slow inactivation kinetics, which lead to extended calcium influx over a long period of time.[36] Increased intracellular calcium in neuronal cell body and dendrites can lead to subsequent alterations in dorsal horn neuron excitability due to calcium-dependent activation of signaling pathways, receptors/ion channels and altered gene transcription. As a consequence, these changes can lead to enhanced excitability of dorsal horn projection neurons, excitatory interneurons, and/or reduced excitability of inhibitory interneurons, which can cause behavioral hypersensitivity, leading to increased pain perception.

Four different isoforms exist for L-type channels ($Ca_V1.1$–$Ca_V1.4$), with $Ca_V1.2$ and $Ca_V1.3$ being strongly expressed in neurons.[37] The biophysical properties of $Ca_V1.3$ differ from other isoforms, in that they are activated faster and at more negative membrane potentials. Thus, $Ca_V1.3$ can contribute to spontaneous neuronal firing.[30] Dysregulation of $Ca_V1.3$ under pain-inducing pathological conditions could thus potentially contribute to enhanced dorsal horn neuron hyperexcitability. The regulation of L-type channel functions in neurons by intracellular signaling molecules has been reviewed recently.[30] It has been shown that regulation of L-type channel α_1-subunits by cAMP-dependent protein kinase or protein kinase A can enhance current activity. In contrast, both calmodulin and calcium binding proteins can regulate calcium-dependent inactivation and calcium-dependent facilitation of Ca_V1 channels.[30,38]

In neuropathic pain models, L-type channels are shown to be dysregulated in DRG and the spinal cord. For example, $Ca_V1.2$ and $Ca_V1.3$ are downregulated in rat DRG neurons following chronic constriction injury of the sciatic nerve,[32] and $Ca_V1.2$ is upregulated in the spinal cord post spinal nerve ligation in a manner that is correlated with behavioral hypersensitivity.[39] $Ca_V1.2$ channels are also expressed within the anterior cingulated cortex, and may be involved in fear learning and behavioral pain responses.[40] Knockdown of the $Ca_V1.2$ L-type channel via intrathecal peptide nucleic acid based anti-sense strategies and small interfering RNA (siRNA) reverses dorsal horn neuron hyperexcitability and behavioral hypersensitivity in the spinal nerve ligated neuropathic pain model, supporting that $Ca_V1.2$ dysregulation may contribute to chronic pain maintenance.[39] These findings contradict previous findings showing that intrathecal administration of L-type channel blockers (verapamil, diltiazem and nimopidine) had no effect on pain behaviors in neuropathic pain models derived from spinal nerve ligation,[41] diabetic and vincristine-induced neuropathies.[42,43] These discrepancies may be due to Ca_V1 subtype selectivity of the agents.

L-type channels are also implicated pharmacologically in morphine-induced analgesia and chronic tolerance. Administration of L-type channel antagonists nifedipine and verapamil enhances morphine analgesia and attenuates the development of morphine tolerance,[44,45] which is in agreement with the notion that increased calcium entry is associated with morphine tolerance.[46] Chronic nimopidine and morphine co-administration has synergistic interactions resulting in an increase in morphine antinociception effects.[45] However, there are some discrepancies regarding the contribution of VGCC subtypes to morphine analgesia and tolerance in biochemical studies. Coadministration of nimopidine and morphine leads to a decrease in $Ca_V1.2$ and an increase in $Ca_V2.2$ channel expression while chronic morphine administration causes an increased expression of both channels in the superficial dorsal horn.[47] Western blot data from another study demonstrate a decreased level of $Ca_V1.3$, but not $Ca_V1.2$ and $Ca_V2.2$ channels in brain stem after chronic morphine treatment.[48] It is likely that morphine analgesia and tolerance are mediated by distinct VGCC subtypes at different locations, which might be distinguished by local treatment with subtype-selective pharmacological agents. Identifying VGCC subtypes underlying morphine analgesia and tolerance with other means would be critical for further advancement in the field.

P/Q-Type Voltage-Gated Calcium Channels

$Ca_V2.1$ P/Q-type channels are expressed at the pre-synaptic terminals in the spinal dorsal horn and may play a role in neurotransmitter release (reviewed in ref. 49). $Ca_V2.1$ is encoded by a single gene, and P- and Q-type channels differ in their ω-agatoxin IVA sensitivity and inactivation kinetics.[50] It is hypothesized that alternative splicing of the $Ca_V2.1$ gene results in the phenotypic variants of P- and Q-type channels, and P-type channels derive from post-translational modifications or modulation of putative proteins.[4,11]

The involvement of P/Q-type channels in pain processing is not well understood. P/Q-type channels show little colocalization with substance P and treatment with ω-agatoxin IVA, a specific inhibitor of P/Q-type channels, has no effect on the release of either substance P or CGRP from peptidergic sensory neurons.[33,51] This suggests that P/Q-type channels are not involved in the release of these pain-inducing neurotransmitters from primary afferents. Instead, P/Q-type channels are highly expressed in dorsal horn laminae II-VI pre-synaptic terminals, where polysynaptic inputs exist.[33] It has been suggested that P/Q-type channels may be involved in the release of excitatory and inhibitory transmitters in spinal dorsal horn.[52,53] Data from

electrophysiology studies have revealed that ω-agatoxin IVA has a minimal effect on monosynaptic C- and Aδ-fiber inputs, but a strong effect on polysynaptic nociceptive transmission.[54] Together, findings from these studies suggest that P/Q-type channels are likely localized on interneurons and play a role in modulating synaptic transmission in the spinal dorsal horn.

In animal models, data regarding the role of $Ca_V2.1$ P/Q-type channels in pain processing are not consistent. In neuropathic pain models, intrathecal administration of ω-agatoxin IVA to block P/Q-type channels has no effect on mechanical allodynia and thermal hyperalgesia.[41,55] Deleting $Ca_V2.1$ in mice results in no change in nociceptive responses to non-injurious noxious thermal stimuli.[56] These data suggest an anti-nociceptive role of P/Q-type channels. However, mice with spontaneously occurring P/Q-type channel mutations show decreased sensitivity to nociceptive stimuli,[57] and deletion of $Ca_V2.1$ in mice results in reduced nociceptive responses in inflammatory and neuropathic pain models.[56] For visceral pain states, Q-type channels are found to be important for acute bladder nociception at the spinal level.[58] Together, it is likely that the role of P/Q-type channels on pain processing depends on the etiology of nociception.

N-Type Voltage-Gated Calcium Channels

$Ca_V2.2$ N-type channels are highly concentrated in neuronal cells including those involved in sensory signal processing such as spinal dorsal horn neurons, dorsal root ganglion cell bodies and their central terminals that form synaptic connections with spinal dorsal horn neurons.[49,59,60] The colocalization of these channels with pain-inducing neurotransmitters[33] and functional blockage of substance P, CGRP and glutamate release by N-type channel antagonists[51,61-63] suggest a major role of the N-type channel in controlling synaptic transmission in pain processing, especially in C- and Aδ-nociceptors. It has been reported that $Ca_V2.2$ N-type channels are upregulated in spinal dorsal horn during the initiation and maintenance stages of pain states after peripheral nerve injury.[64] Thus, blocking synaptic transmission via N-type channels could serve as a prime target for reducing pain signal transmission to the central nervous system.

Small peptide inhibitors of N-type channels from cone snail toxins have been used to study the functional roles of these channels in pain processing. ω-conotoxins MVIIA (SNX-111, ziconotide or Prialt) and GVIA were used to examine their interaction with specific gating states of N-type channels.[65] Originally, ω-conotoxins are thought to block the N-type channel pores completely by binding to amino acid residues just outside the pore to diminish calcium influx,[66] which may lead to adverse side effects. Recent studies, however, show that ω-conotoxin GVIA is able to modulate the gating properties of N-type channels, thereby leading to a reduction in action potential-induced calcium influx by ~50% without blocking the pore.[67] In addition, ω-conotoxin GVIA binding can destabilize the open state and alter gating transitions between closed states of N-type channels,[67,68] which can reduce pain-inducing neurotransmitter release in the dorsal horn. This is in contrast with findings that ω-conotoxin MVIIA blocks all gating states.[69] This discrepancy may derive from the differences between the secondary binding sites for these toxins.[69] Thus, modulation of N-type channels by ω-conotoxin GVIA and its derivatives may provide a proof of concept for the development of new state-dependent N-type VGCC blockers for pain management.

Omega-conotoxin MVIIA (Ziconotide or Prialt, Elan Pharmaceuticals Inc., San Diego, CA) is the first N-type channel antagonist approved by the US Food and Drug Administration and European Medicines Agency for management of chronic severe pain refractory to other current pain medications. Data from pre-clinical studies have shown that intrathecal administration of ω-conotoxin MVIIA inhibits hyperalgesia and allodynia in neuropathic and inflammatory pain models.[70] Due to the wide-distribution of N-type channels and the peptidergic nature of the drug, its application is limited to intrathecal delivery (reviewed in ref. 71). A recent study introduced a new small molecule, N-triazole oxindole TROX-1, an inhibitor of $Ca_V2.2$ N-type channels that can be administered orally. TROX-1 is able to reverse inflammatory-induced hyperalgesia and nerve-injury induced allodynia to the same extent as current anti-inflammatory and neuropathic pain drugs.[72] A substantial effort has been made towards developing small molecule N-type channel blockers that may be efficacious in pain management post systemic administration (reviewed in ref. 73).

Modulation of N-type channels for sensory information processing can occur through voltage-dependent inhibition by the Gβγ subunit of G-proteins, and voltage-independent inhibition by protein tyrosine kinase in DRG neurons. Inhibition of N-type channel currents upon Gβγ binding can be reversed by protein kinase C-dependent phosphorylation of the Gβγ binding site of the N-type VGCC.[28,74,75] Interestingly, activation of μ-opioid receptors results in inhibition of N-type channels through Gβγ subunits.[76,77] Similar activation of ORL1 (opioid receptor-like 1) receptors (also known as nociceptin receptors) has also been shown to inhibit N-type channels through a G-protein mediated mechanism in the absence of the ligand nociceptin.[78] In addition, ORL1 receptors have been shown to heterodimerize with μ-opioid receptors and associate with N-type channels, resulting in internalization of N-type channels. By doing so, the ORL1 receptor appears to act as a physical link between μ-opioid receptors and N-type channels to modulate opioid receptor mediated regulation of channel activity and trafficking.[79] Prolonged exposure to the ORL1 receptor agonist nociceptin leads to protein kinase C-dependent internalization of N-type channel complexes and consequently downregulated calcium entry, a regulatory means that could have significant implication in controlling N-type channel functions in sensory pathway.[80]

Identification of alternative splicing of the N-type VGCC α_1-subunit, such as exon37a and exon37b splice variants, may lead to improvement of drug specificity for modulating N-type channel activity in pain processing. Both exon37a and exon37b are mutually exclusive and encode 32 amino acids of the proximal c-terminal region of the N-type channel that differ by 14 amino acids.[5,81] Exon37a has been shown to be almost exclusively expressed in capsaicin-sensitive nociceptive DRG neurons and support increased N-type current densities.[5,82] Specifically,

exon37a containing channels remain open longer upon activation compared to those containing exon37b.[5,82] Silencing exon37a via siRNA in vivo reduces basal thermal nociception and development of thermal and mechanical hyperalgesia during inflammatory and neuropathic pain states.[3] Voltage-dependent G protein inhibition of N-type channels is indistinguishable between exon37a and exon37b isoforms.[24,83] However, exon37a appears to confer a greater susceptibility to voltage-independent inhibition of N-type channel currents through a mechanism involving Gi/o subunits and kinase-dependent phosphorylation.[9,24] A tyrosine encoded within exon37a, but not exon37b, acts as a molecular switch in controlling N-type channel current density and voltage-independent inhibition that ultimately leads to modulation of nociception.[24] Furthermore, exon37a regulates the extent of μ-opioid receptor-mediated inhibition of N-type channels, and the absence of exon37a results in reduced morphine-induced analgesia without affecting basal response to noxious thermal stimuli.[81]

R-Type Voltage-Gated Calcium Channels

$Ca_V2.3$ R-type channels are classified as being "resistant" to inhibitors of other high-voltage-activated L-, N-, P- and Q-type channels. The R-type channels are found in neuronal cells and may play a role in regulating neurotransmitter release and neuronal excitability.[84,85] $Ca_V2.3$ channels have been suggested to contribute to pain transmission by regulating both nociceptive and anti-nociceptive behaviors through spinal and supraspinal mechanisms as shown in mutant mice lacking the $Ca_V2.3$ R-type channels.[86] Data from a recent study have shown that SNX-482, a selective R-type channel antagonist from tarantula venom, inhibits C- and Aδ-fiber-mediated dorsal horn neuronal responses and neuropathic pain states in nerve-injured rats, suggesting that R-type channels may contribute to central sensitization in the spinal cord during neuropathic pain processing.[87] This is supported by data from a tissue injury model in which SNX-482 treatment increased behavioral sensitivity during the first phase of formalin-induced pain response but produced analgesic effects in the second phase of the formalin-test, which is considered a centrally mediated nociceptive response.[88]

T-Type Voltage-Gated Calcium Channels

Unlike Ca_V1 and Ca_V2 channels, Ca_V3 T-type channels activate at hyperpolarized levels, close to resting potentials (low voltage thresholds). The T-type channels are expressed in tissues throughout the body, including the heart, smooth muscles, pancreas, kidney and neuronal tissues.[35,89-93] It appears that T-type channels consist of α_1-subunits that do not associate with auxiliary subunits.[94,95] In sensory pathways, T-type channels are located on primary afferent terminals and dorsal root ganglia, with $Ca_V3.2$ being the most abundant isoform in the DRG, and thus the T-type channel subtype capable of the most prominent role in nociception.[96-98] Mechanisms underlying T-type channel regulation and neuronal functions have been reviewed recently.[21,99] Data from electrophysiology studies suggest that T-type channels are involved in shaping action potentials, regulating neuronal firing patterns, lowering action potential thresholds, promoting burst firing, oscillatory behavior and enhancing synaptic excitation.[21] T-type channel activation close to the resting potential allows calcium influx in response to sub-threshold synaptic input when the cells are at rest, and enhances the neuronal excitability by boosting synaptic inputs and lowering the threshold for high-threshold spike generation.[100-102] In addition, Ca_V3 T-type channels form complexes with low-voltage-activated A-type potassium channels, allowing the potassium channels to regulate neuronal firing at a subthreshold membrane potential range.[103] Thus, blocking the T-type channel can lead to overall reduction of neuronal excitability.

Data from animal studies suggest that functional contribution of T-type channels to pain processing varies based on their modalities, subtypes and anatomical locations. Increased T-type channel currents are found in small DRG neurons following chronic constriction injury of the sciatic nerve,[104] but in medium-size DRG neurons following chemical-induced diabetic neuropathy.[105] These changes may lead to increased excitability (lowered activation threshold) of sensory neurons that can contribute to the pathological pain responses such as mechanical alloydynia and thermal hyperalgesia observed in both models. T-type channels are implicated in the development of chronic musculoskeletal pain syndromes, as mice deficient in $Ca_V3.2$ fail to develop acid-induced chronic mechanical hyperalgesia.[106] Moreover, mice lacking $Ca_V3.2$ show a hypoalgesic response to acute, somatic, visceral and tonic inflammatory insults, altogether suggesting that $Ca_V3.2$ T-type channels play a pronociceptive role in processing of noxious stimulation.[107] This is further supported by data indicating that $Ca_V3.2$ expression is increased in DRG in diabetic neuropathy and mechanical nerve injury models,[108,109] and knock down of $Ca_V3.2$ by siRNA or antisense oligonucleotides results in anti-nociceptive effects in these pain models.[96,108,110] In addition, $Ca_V3.1$ deficient mice show a reduction in nerve injury-induced behavioral hypersensitivity, suggesting that $Ca_V3.1$ may also be a contributor to neuropathic pain processing.[111] However, $Ca_V3.1$ deletion in mice leads to an increase in visceral pain, similar to what is observed following thalamic infusion of T-type channel blockers. This suggests that $Ca_V3.1$ T-type channels in the thalamus are anti-nociceptive.[112]

Since T-type channels are subjected to dysregulation under some pain-inducing conditions, normalizing dysregulated T-type channels thus represents an attractive alternative strategy in developing novel pain medications. It has been reported that inhibiting T-type channels using non-selective T-type channel antagonists such as ethosuximide and mibefradil, effectively blocks and reverses both tactile hypersensitivity and thermal hyperalgesia in pain models.[113,114] These non-selective antagonists inhibit input spikes, indicative of diminished synaptic activity, probably through a reduction in exocytosis of neurotransmitter from primary afferent neurons.[113] Furthermore, $Ca_V3.2$ T-type channel-dependent activation of extracellular signal-regulated kinase (ERK) in the anterior nucleus of paraventricular thalamus correlates with acid-induced chronic hyperalgesia, and inhibiting ERK activation in wild-type mice prevents chronic mechanical

hyperalgesia.[106] The endogenous reducing agent, L-cysteine, selectively and potently enhances T-type channel currents and promotes cutaneous thermal and mechanical hyperalgesia. In contrast, the oxidizing agent, 5,5'-dithio-bis-(2-nitrobenzoic acid), inhibits T-type channel currents in small dorsal root ganglia and alleviates hyperalgesia in pain models.[115,116] Hydrogen sulfide, a gasotransmitter, has also been implicated as a neuromodulator in sensory transmission by activating $Ca_V3.2$ channels in primary afferents and in spinal nociceptive neurons, thus leading to sensitization of nociceptive processing and hyperalgesia.[117] Treatment with inhibitors of T-type channels or of cystathionine-γ-lyase (CSE), an enzyme involved in hydrogen sulfide formation, causes a reversal of hyperalgesia and allodynia in spinal nerve injured rats that show an upregulation of $Ca_V3.2$, but not CSE, in injured DRG. This suggests that endogenous hydrogen sulfide may activate or sensitize elevated T-type channels that contribute to neuropathic pain maintenance.[108]

VGCC $\alpha_2\delta$-1 Proteins as the Binding Site for Gabapentinoids

The $\alpha_2\delta$-1 subunit of high VGCC is the binding site for gabapentin and pregabalin, another class of drugs with pain relief properties in preclinical and clinical studies. These gabapentinoid drugs are not calcium channel blockers, but may play an important role in normalizing VGCC malfunctions in disease states such as pain and epilepsy. The direct actions of gabapentinoids on ion channels are reviewed by Dr. Osvaldo Uchitel in this issue.[118] Recently, a panel of experts recommended these gabapentinoid drugs as the first line treatment for neuropathic pain conditions.[119] Even though these drugs were designed to mimic the structure of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), later studies have found that their actions are not mainly mediated through the GABAergic system (reviewed in ref. 120). Since expression of $\alpha_2\delta$-1 proteins is dysregulated in DRG and spinal dorsal horn in neuropathic pain models,[121-125] uncovering their contributions to pain processing and the actions of these drugs in pain relief is critically important. Data from biochemical studies have supported that increased expression of this subunit leads to increased calcium channel currents in DRG neurons, dorsal horn neuron hyperexcitability and behavioral hypersensitivity. Gabapentin can normalize these abnormal behaviors without affecting normal activities in the control groups. This supports that dysregulation of $\alpha_2\delta$-1 proteins in the sensory pathway contributes to dorsal horn neuron hyperexcitability and pain processing, and that the actions of gabapentin in pain relief are mediated by normalizing these maladaptive changes.[123,125-127] In contrast, upregulated $\alpha_2\delta$-1 proteins may interfere with the antinociceptive effects of ω-conotoxins.[128] At the peripheral level, it is noteworthy that calcium currents are actually reduced in DRG neurons from nerve-injured rats that show DRG $\alpha_2\delta$-1 upregulation and behavioral hypersensitivity.[123,129-131] This contradicts data from $\alpha_2\delta$-1 overexpressing transgenic mice in which increased calcium currents in isolated DRG neurons correlate with behavioral hypersensitivity.[126] This discrepancy may be due to additional modulatory effects from other injury factor(s) that are missing in the $\alpha_2\delta$-1 overexpressing mice (without injury). At the spinal level, it appears that elevated $\alpha_2\delta$-1 mediates behavioral hypersensitivity through enhanced excitatory pre-synaptic input that activates glutamate receptors at post-synaptic dorsal horn neurons.[131] The mechanism of this neuroplasticity in pain processing is not yet clear, but data from recent studies have suggested that injury-induced increase of $\alpha_2\delta$-1 in DRG leads to increased trafficking of $\alpha_2\delta$-1 proteins to the pre-synaptic terminals that may cause increased VGCC expression and VGCC-mediated neurotransmission.[121,122,133,134] This process may ultimately lead to abnormal synaptogenesis.[135,136] Since both processes are sensitive to blockade by gabapentinoids,[121,133,136] normalizing these cellular maladaptive changes in pain-inducing conditions may underlie a chronic mechanism of gabapentinoid drugs in pain relief.

Summary

VGCCs are widely expressed and distributed throughout the body and play an obligatory role in important physiological functions. The specificity of VGCC functions derives from tissue-specific distribution of VGCC subtypes, their coupling to unique intracellular signaling pathways and interactions with other proteins, that are critical in mediating cellular functions. Preclinical data have implicated the involvement of dysregulation and/or malfunctions of VGCC subtypes in pain processing. This includes, but is not limited to, changes in expression and regulation of VGCC subtypes/subunits, and alterations in functional interactions of VGCC subtypes with other proteins or cellular molecules/co-factors that directly or indirectly modulate abnormal VGCC functions in pain pathways. Thus, normalizing these pathological maladaptive changes in different pain-inducing etiologies represents an attractive approach for designing the next generation of pain medications that should be more target-specific and have fewer side effects. This could also be achieved by modifying existing VGCC drugs to improve their efficacy and toxicity profiles. As discussed in a recent review in reference 137, some of these drugs are in the pipeline and our better understanding of VGCC functions in pain processing will eventually lead to more promising pain medications.

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References

1. Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol Rev 2005; 57:411-25.
2. Perez-Reyes E. Molecular physiology of low-voltage-activated t-type calcium channels. Physiol Rev 2003; 83:117-61.
3. Altier C, Dale CS, Kisilevsky AE, Chapman K, Castiglioni AJ, Matthews EA, et al. Differential role of N-type calcium channel splice isoforms in pain. J Neurosci 2007; 27:6363-73.
4. Bourinet E, Soong TW, Sutton K, Slaymaker S, Mathews E, Monteil A, et al. Splicing of α1A subunit gene generates phenotypic variants of P- and Q-type calcium channels. Nat Neurosci 1999; 2:407-15.
5. Castiglioni AJ, Raingo J, Lipscombe D. Alternative splicing in the C-terminus of $Ca_V2.2$ controls expression and gating of N-type calcium channels. J Physiol 2006; 576:119-34.

6. Lin Y, McDonough SI, Lipscombe D. Alternative splicing in the voltage-sensing region of N-Type $Ca_V2.2$ channels modulates channel kinetics. J Neurophysiol 2004; 92:2820-30.
7. Lin Z, Haus S, Edgerton J, Lipscombe D. Identification of functionally distinct isoforms of the N-type Ca^{2+} channel in rat sympathetic ganglia and brain. Neuron 1997; 18:153-66.
8. Lin Z, Lin Y, Schorge S, Pan JQ, Beierlein M, Lipscombe D. Alternative splicing of a short cassette exon in α1B generates functionally distinct N-type calcium channels in central and peripheral neurons. J Neurosci 1999; 19:5322-31.
9. Lipscombe D, Raingo J. Alternative splicing matters: N-type calcium channels in nociceptors. Channels (Austin) 2007; 1:225-7.
10. Rajapaksha WR, Wang D, Davies JN, Chen L, Zamponi GW, Fisher TE. Novel splice variants of rat $Ca_V2.1$ that lack much of the synaptic protein interaction site are expressed in neuroendocrine cells. J Biol Chem 2008; 283:15997-6003.
11. Tsunemi T, Saegusa H, Ishikawa K, Nagayama S, Murakoshi T, Mizusawa H, Tanabe T. Novel $Ca_V2.1$ splice variants isolated from Purkinje cells do not generate P-type Ca^{2+} current. J Biol Chem 2002; 277:7214-21.
12. Yu FH, Yarov-Yarovoy V, Gutman GA, Catterall WA. Overview of molecular relationships in the voltage-gated ion channel superfamily. Pharmacol Rev 2005; 57:387-95.
13. Karunasekara Y, Dulhunty AF, Casarotto MG. The voltage-gated calcium-channel β subunit: more than just an accessory. Eur Biophys J 2009; 39:75-81.
14. Hidalgo P, Neely A. Multiplicity of protein interactions and functions of the voltage-gated calcium channel beta-subunit. Cell Calcium 2007; 42:389-96.
15. Flynn R, Zamponi G. Calcium channel regulation by RGK proteins. Channels (Austin) 2010; 4:434-9.
16. Ellis SB, Williams ME, Ways NR, Brenner R, Sharp AH, Leung AT, et al. Sequence and expression of mRNAs encoding the α1 and α2 subunits of a DHP-sensitive calcium channel. Science 1988; 241:1661-4.
17. De Jongh KS, Warner C, Catterall WA. Subunits of purified calcium channels. Alpha2 and δ are encoded by the same gene. J Biol Chem 1990; 265:14738-41.
18. Davies A, Hendrich J, Van Minh AT, Wratten J, Douglas L, Dolphin AC. Functional biology of the $\alpha_2\delta$ subunits of voltage-gated calcium channels. Trends Pharmacol Sci 2007; 28:220-8.
19. Klugbauer N, Marais E, Hofmann F. Calcium channel α2δ subunits: differential expression, function and drug binding. J Bioenerg Biomembr 2003; 35:639-47.
20. Dolphin AC. Calcium channel diversity: multiple roles of calcium channel subunits. Curr Opin Neurobiol 2009; 19:237-44.
21. Iftinca MC, Zamponi GW. Regulation of neuronal T-type calcium channels. Trends Pharmacol Sci 2009; 30:32-40.
22. Lacinova L, Klugbauer N, Hofmann F. State- and isoform-dependent interaction of isradipine with the α1C L-type calcium channel. Pflugers Arch 2000; 440:50-60.
23. Mich PM, Horne WA. Alternative splicing of the Ca^{2+} channel β4 subunit confers specificity for gabapentin inhibition of $Ca_V2.1$ trafficking. Mol Pharmacol 2008; 74:904-12.
24. Raingo J, Castiglioni AJ, Lipscombe D. Alternative splicing controls G protein-dependent inhibition of N-type calcium channels in nociceptors. Nat Neurosci 2007; 10:285-92.
25. Szabo Z, Obermair GJ, Cooper CB, Zamponi GW, Flucher BE. Role of the synprint site in presynaptic targeting of the calcium channel $Ca_V2.2$ in hippocampal neurons. Eur J Neurosci 2006; 24:709-18.
26. Thaler C, Gray AC, Lipscombe D. Cumulative inactivation of N-type $Ca_V2.2$ calcium channels modified by alternative splicing. Proc Natl Acad Sci USA 2004; 101:5675-9.
27. Vendel AC, Terry MD, Striegel AR, Iverson NM, Leuranguer V, Rithner CD, et al. Alternative splicing of the voltage-gated Ca^{2+} channel β4 subunit creates a uniquely folded N-terminal protein binding domain with cell-specific expression in the cerebellar cortex. J Neurosci 2006; 26:2635-44.
28. Catterall WA. Structure and regulation of voltage-gated Ca^{2+} channels. Annu Rev Cell Dev Biol 2000; 16:521-55.
29. Perez-Reyes E. G protein-mediated inhibition of $Ca_V3.2$ T-type channels revisited. Mol Pharmacol 2010; 77:136-8.
30. Calin-Jageman I, Lee A. Ca_V1 L-type Ca^{2+} channel signaling complexes in neurons. J Neurochem 2008; 105:573-83.
31. Dolmetsch RE, Pajvani U, Fife K, Spotts JM, Greenberg ME. Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. Science 2001; 294:333-9.
32. Kim DS, Yoon CH, Lee SJ, Park SY, Yoo HJ, Cho HJ. Changes in voltage-gated calcium channel α1 gene expression in rat dorsal root ganglia following peripheral nerve injury. Brain Res Mol Brain Res 2001; 96:151-6.
33. Westenbroek RE, Hoskins L, Catterall WA. Localization of Ca^{2+} channel subtypes on rat spinal motor neurons, interneurons and nerve terminals. J Neurosci 1998; 18:6319-30.
34. Hell JW, Westenbroek RE, Warner C, Ahlijanian MK, Prystay W, Gilbert MM, et al. Identification and differential subcellular localization of the neuronal class C and class D L-type calcium channel α1 subunits. J Cell Biol 1993; 123:949-62.
35. Trimmer JS, Rhodes KJ. Localization of voltage-gated ion channels in mammalian brain. Annu Rev Physiol 2004; 66:477-519.
36. Nowycky MC, Fox AP, Tsien RW. Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature 1985; 316:440-3.
37. Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, et al. Nomenclature of voltage-gated calcium channels. Neuron 2000; 25:533-5.
38. Hell JW, Yokoyama CT, Wong ST, Warner C, Snutch TP, Catterall WA. Differential phosphorylation of two size forms of the neuronal class C L-type calcium channel α1 subunit. J Biol Chem 1993; 268:19451-7.
39. Fossat P, Dobremez E, Bouali-Benazzouz R, Favereaux A, Bertrand SS, Kilk K, et al. Knockdown of L calcium channel subtypes: differential effects in neuropathic pain. J Neurosci 2010; 30:1073-85.
40. Jeon D, Kim S, Chetana M, Jo D, Ruley HE, Lin SY, et al. Observational fear learning involves affective pain system and $Ca_V1.2$ Ca^{2+} channels in ACC. Nat Neurosci 2010; 13:482-8.
41. Chaplan SR, Pogrel JW, Yaksh TL. Role of voltage-dependent calcium channel subtypes in experimental tactile allodynia. J Pharmacol Exp Ther 1994; 269:1117-23.
42. Calcutt NA, Chaplan SR. Spinal pharmacology of tactile allodynia in diabetic rats. Br J Pharmacol 1997; 122:1478-82.
43. Fukuizumi T, Ohkubo T, Kitamura K. Spinally delivered N-, P/Q- and L-type Ca^{2+}-channel blockers potentiate morphine analgesia in mice. Life Sci 2003; 73:2873-81.
44. Michaluk J, Karolewicz B, Antkiewicz-Michaluk L, Vetulani J. Effects of various Ca^{2+} channel antagonists on morphine analgesia, tolerance and dependence, and on blood pressure in the rat. Eur J Pharmacol 1998; 352:189-97.
45. Ray SB, Mishra P, Verma D, Gupta A, Wadhwa S. Nimodipine is more effective than nifedipine in attenuating morphine tolerance on chronic co-administration in the rat tail-flick test. Indian J Exp Biol 2008; 46:219-28.
46. Welch SP, Olson KG. Opiate tolerance-induced modulation of free intracellular calcium in synaptosomes. Life Sci 1991; 48:1853-61.
47. Verma D, Gupta YK, Parashar A, Ray SB. Differential expression of L- and N-type voltage-sensitive calcium channels in the spinal cord of morphine + nimodipine treated rats. Brain Res 2009; 1249:128-34.
48. Haller VL, Bernstein MA, Welch SP. Chronic morphine treatment decreases the $Ca_V1.3$ subunit of the L-type calcium channel. Eur J Pharmacol 2008; 578:101-7.
49. Catterall WA, Few AP. Calcium channel regulation and presynaptic plasticity. Neuron 2008; 59:882-901.
50. Tottene A, Moretti A, Pietrobon D. Functional diversity of P-type and R-type calcium channels in rat cerebellar neurons. J Neurosci 1996; 16:6353-63.
51. Evans AR, Nicol GD, Vasko MR. Differential regulation of evoked peptide release by voltage-sensitive calcium channels in rat sensory neurons. Brain Res 1996; 712:265-73.
52. Araque A, Clarac F, Buno W. P-type Ca^{2+} channels mediate excitatory and inhibitory synaptic transmitter release in crayfish muscle. Proc Natl Acad Sci USA 1994; 91:4224-8.
53. Takahashi T, Momiyama A. Different types of calcium channels mediate central synaptic transmission. Nature 1993; 366:156-8.
54. Heinke B, Balzer E, Sandkuhler J. Pre- and postsynaptic contributions of voltage-dependent Ca^{2+} channels to nociceptive transmission in rat spinal lamina I neurons. Eur J Neurosci 2004; 19:103-11.
55. Yamamoto T, Sakashita Y. Differential effects of intrathecally administered N- and P-type voltage-sensitive calcium channel blockers upon two models of experimental mononeuropathy in the rat. Brain Res 1998; 794:329-32.
56. Luvisetto S, Marinelli S, Panasiti MS, D'Amato FR, Fletcher CF, Pavone F, Pietrobon D. Pain sensitivity in mice lacking the $Ca_V2.1\alpha1$ subunit of P/Q-type Ca^{2+} channels. Neuroscience 2006; 142:823-32.
57. Fukumoto N, Obama Y, Kitamura N, Niimi K, Takahashi E, Itakura C, Shibuya I. Hypoalgesic behaviors of P/Q-type voltage-gated Ca^{2+} channel mutant mouse, rolling mouse Nagoya. Neuroscience 2009; 160:165-73.
58. Su X, Leon LA, Laping NJ. Role of spinal $Ca_V2.2$ and $Ca_V2.1$ ion channels in bladder nociception. J Urol 2008; 179:2464-9.
59. Westenbroek RE, Hell JW, Warner C, Dubel SJ, Snutch TP, Catterall WA. Biochemical properties and subcellular distribution of an N-type calcium channel α1 subunit. Neuron 1992; 9:1099-115.
60. Gohil K, Bell JR, Ramachandran J, Miljanich GP. Neuroanatomical distribution of receptors for a novel voltage-sensitive calcium-channel antagonist, SNX-230 (omega-conopeptide MVIIC). Brain Res 1994; 653:258-66.
61. Santicioli P, Del Bianco E, Tramontana M, Geppetti P, Maggi CA. Release of calcitonin gene-related peptide like-immunoreactivity induced by electrical field stimulation from rat spinal afferents is mediated by conotoxin-sensitive calcium channels. Neurosci Lett 1992; 136:161-4.
62. Maggi CA, Tramontana M, Cecconi R, Santicioli P. Neurochemical evidence for the involvement of N-type calcium channels in transmitter secretion from peripheral endings of sensory nerves in guinea pigs. Neurosci Lett 1990; 114:203-6.
63. McGivern JG, McDonough SI. Voltage-gated calcium channels as targets for the treatment of chronic pain. Curr Drug Targets CNS Neurol Disord 2004; 3:457-78.
64. Cizkova D, Marsala J, Lukacova N, Marsala M, Jergova S, Orendacova J, Yaksh TL. Localization of N-type Ca^{2+} channels in the rat spinal cord following chronic constrictive nerve injury. Exp Brain Res 2002; 147:456-63.
65. Olivera BM, Miljanich GP, Ramachandran J, Adams ME. Calcium channel diversity and neurotransmitter release: the omega-conotoxins and omega-agatoxins. Annu Rev Biochem 1994; 63:823-67.

66. Feng ZP, Hamid J, Doering C, Bosey GM, Snutch TP, Zamponi GW. Residue Gly1326 of the N-type calcium channel α1B subunit controls reversibility of omega-conotoxin GVIA and MVIIA block. J Biol Chem 2001; 276:15728-35.
67. Yarotskyy V, Elmslie KS. omega-conotoxin GVIA alters gating charge movement of N-type ($Ca_V2.2$) calcium channels. J Neurophysiol 2009; 101:332-40.
68. Yarotskyy V, Elmslie KS. Interference between two modulators of N-type ($Ca_V2.2$) calcium channel gating demonstrates that omega-conotoxin GVIA disrupts open state gating. Biochim Biophys Acta 2010; 1798:1821-8.
69. Feng ZP, Doering CJ, Winkfein RJ, Beedle AM, Spafford JD, Zamponi GW. Determinants of inhibition of transiently expressed voltage-gated calcium channels by omega-conotoxins GVIA and MVIIA. J Biol Chem 2003; 278:20171-8.
70. Miljanich GP. Ziconotide: neuronal calcium channel blocker for treating severe chronic pain. Curr Med Chem 2004; 11:3029-40.
71. Schmidtko A, Lotsch J, Freynhagen R, Geisslinger G. Ziconotide for treatment of severe chronic pain. Lancet 2010; 375:1569-77.
72. Abbadie C, McManus OB, Sun SY, Bugianesi RM, Dai G, Haedo RJ, et al. Analgesic effects of a substituted N-triazole oxindole (TROX-1), a state-dependent, voltage-gated calcium channel 2 blocker. J Pharmacol Exp Ther 2010; 334:545-55.
73. Yamamoto T, Takahara A. Recent updates of N-type calcium channel blockers with therapeutic potential for neuropathic pain and stroke. Curr Top Med Chem 2009; 9:377-95.
74. Swartz KJ. Modulation of Ca^{2+} channels by protein kinase C in rat central and peripheral neurons: disruption of G protein-mediated inhibition. Neuron 1993; 11:305-20.
75. Zamponi GW, Bourinet E, Nelson D, Nargeot J, Snutch TP. Crosstalk between G proteins and protein kinase C mediated by the calcium channel α1 subunit. Nature 1997; 385:442-6.
76. Bourinet E, Soong TW, Stea A, Snutch TP. Determinants of the G protein-dependent opioid modulation of neuronal calcium channels. Proc Natl Acad Sci USA 1996; 93:1486-91.
77. Seward E, Hammond C, Henderson G. Mu-opioid-receptor-mediated inhibition of the N-type calcium-channel current. Proc Biol Sci 1991; 244:129-35.
78. Beedle AM, McRory JE, Poirot O, Doering CJ, Altier C, Barrere C, et al. Agonist-independent modulation of N-type calcium channels by ORL1 receptors. Nat Neurosci 2004; 7:118-25.
79. Evans RM, You H, Hameed S, Altier C, Mezghrani A, Bourinet E, Zamponi GW. Heterodimerization of ORL1 and opioid receptors and its consequences for N-type calcium channel regulation. J Biol Chem 2010; 285:1032-40.
80. Altier C, Khosravani H, Evans RM, Hameed S, Peloquin JB, Vartian BA, et al. ORL1 receptor-mediated internalization of N-type calcium channels. Nat Neurosci 2006; 9:31-40.
81. Andrade A, Denome S, Jiang YQ, Marangoudakis S, Lipscombe D. Opioid inhibition of N-type Ca^{2+} channels and spinal analgesia couple to alternative splicing. Nat Neurosci 2010; 13:1249-56.
82. Bell TJ, Thaler C, Castiglioni AJ, Helton TD, Lipscombe D. Cell-specific alternative splicing increases calcium channel current density in the pain pathway. Neuron 2004; 41:127-38.
83. Ikeda SR. Voltage-dependent modulation of N-type calcium channels by G-protein beta-γ subunits. Nature 1996; 380:255-8.
84. Dietrich D, Kirschstein T, Kukley M, Pereverzev A, von der Brelie C, Schneider T, Beck H. Functional specialization of presynaptic $Ca_V2.3$ Ca^{2+} channels. Neuron 2003; 39:483-96.
85. Brown SP, Safo PK, Regehr WG. Endocannabinoids inhibit transmission at granule cell to Purkinje cell synapses by modulating three types of presynaptic calcium channels. J Neurosci 2004; 24:5623-31.
86. Saegusa H, Kurihara T, Zong S, Minowa O, Kazuno A, Han W, et al. Altered pain responses in mice lacking α1E subunit of the voltage-dependent Ca^{2+} channel. Proc Natl Acad Sci USA 2000; 97:6132-7.
87. Matthews EA, Bee LA, Stephens GJ, Dickenson AH. The $Ca_V2.3$ calcium channel antagonist SNX-482 reduces dorsal horn neuronal responses in a rat model of chronic neuropathic pain. Eur J Neurosci 2007; 25:3561-9.
88. Murakami M, Nakagawasai O, Suzuki T, Mobarakeh II, Sakurada Y, Murata A, et al. Antinociceptive effect of different types of calcium channel inhibitors and the distribution of various calcium channel α1 subunits in the dorsal horn of spinal cord in mice. Brain Res 2004; 1024:122-9.
89. Cribbs LL, Lee JH, Yang J, Satin J, Zhang Y, Daud A, et al. Cloning and characterization of α1H from human heart, a member of the T-type Ca^{2+} channel gene family. Circ Res 1998; 83:103-9.
90. Monteil A, Chemin J, Bourinet E, Mennessier G, Lory P, Nargeot J. Molecular and functional properties of the human α1G subunit that forms T-type calcium channels. J Biol Chem 2000; 275:6090-100.
91. Molineux ML, McRory JE, McKay BE, Hamid J, Mehaffey WH, Rehak R, et al. Specific T-type calcium channel isoforms are associated with distinct burst phenotypes in deep cerebellar nuclear neurons. Proc Natl Acad Sci USA 2006; 103:5555-60.
92. Vassort G, Talavera K, Alvarez JL. Role of T-type Ca^{2+} channels in the heart. Cell Calcium 2006; 40:205-20.
93. Fry CH, Sui G, Wu C. T-type Ca^{2+} channels in non-vascular smooth muscles. Cell Calcium 2006; 40:231-9.
94. Lambert RC, Maulet Y, Mouton J, Beattie R, Volsen S, De Waard M, Feltz A. T-type Ca^{2+} current properties are not modified by Ca^{2+} channel β subunit depletion in nodosus ganglion neurons. J Neurosci 1997; 17:6621-8.
95. Lacinova L, Klugbauer N, Hofmann F. Absence of modulation of the expressed calcium channel α1G subunit by $\alpha_2\delta$ subunits. J Physiol 1999; 516:639-45.
96. Bourinet E, Alloui A, Monteil A, Barrere C, Couette B, Poirot O, et al. Silencing of the $Ca_V3.2$ T-type calcium channel gene in sensory neurons demonstrates its major role in nociception. EMBO J 2005; 24:315-24.
97. Scroggs RS, Fox AP. Calcium current variation between acutely isolated adult rat dorsal root ganglion neurons of different size. J Physiol 1992; 445:639-58.
98. Shin JB, Martinez-Salgado C, Heppenstall PA, Lewin GR. A T-type calcium channel required for normal function of a mammalian mechanoreceptor. Nat Neurosci 2003; 6:724-30.
99. Huc S, Monteil A, Bidaud I, Barbara G, Chemin J, Lory P. Regulation of T-type calcium channels: signalling pathways and functional implications. Biochim Biophys Acta 2009; 1793:947-52.
100. Amir R, Michaelis M, Devor M. Burst discharge in primary sensory neurons: triggered by subthreshold oscillations, maintained by depolarizing afterpotentials. J Neurosci 2002; 22:1187-98.
101. Huguenard JR. Low-threshold calcium currents in central nervous system neurons. Annual Review of Physiology 1996; 58:329-48.
102. Matthews EA, Dickenson AH. Effects of ethosuximide, a T-type Ca^{2+} channel blocker, on dorsal horn neuronal responses in rats. Eur J Pharmacol 2001; 415:141-9.
103. Anderson D, Mehaffey WH, Iftinca M, Rehak R, Engbers JD, Hameed S, et al. Regulation of neuronal activity by Ca_V3-K_V4 channel signaling complexes. Nat Neurosci 2010; 13:333-7.
104. Jagodic MM, Pathirathna S, Joksovic PM, Lee W, Nelson MT, Naik AK, et al. Upregulation of the T-type calcium current in small rat sensory neurons after chronic constrictive injury of the sciatic nerve. J Neurophysiol 2008; 99:3151-6.
105. Jagodic MM, Pathirathna S, Nelson MT, Mancuso S, Joksovic PM, Rosenberg ER, et al. Cell-specific alterations of T-type calcium current in painful diabetic neuropathy enhance excitability of sensory neurons. J Neurosci 2007; 27:3305-16.
106. Chen WK, Liu IY, Chang YT, Chen YC, Chen CC, Yen CT, Shin HS. $Ca_V3.2$ T-type Ca^{2+} channel-dependent activation of ERK in paraventricular thalamus modulates acid-induced chronic muscle pain. J Neurosci 2010; 30:10360-8.
107. Choi S, Na HS, Kim J, Lee J, Lee S, Kim D, et al. Attenuated pain responses in mice lacking $Ca_V3.2$ T-type channels. Genes Brain Behav 2007; 6:425-31.
108. Takahashi T, Aoki Y, Okubo K, Maeda Y, Sekiguchi F, Mitani K, et al. Upregulation of $Ca_V3.2$ T-type calcium channels targeted by endogenous hydrogen sulfide contributes to maintenance of neuropathic pain. Pain 2010; 150:183-91.
109. Latham JR, Pathirathna S, Jagodic MM, Choe WJ, Levin ME, Nelson MT, et al. Selective T-type calcium channel blockade alleviates hyperalgesia in ob/ob mice. Diabetes 2009; 58:2656-65.
110. Messinger RB, Naik AK, Jagodic MM, Nelson MT, Lee WY, Choe WJ, et al. In vivo silencing of the $Ca_V3.2$ T-type calcium channels in sensory neurons alleviates hyperalgesia in rats with streptozocin-induced diabetic neuropathy. Pain 2009; 145:184-95.
111. Na HS, Choi S, Kim J, Park J, Shin HS. Attenuated neuropathic pain in $Ca_V3.1$ null mice. Mol Cells 2008; 25:242-6.
112. Kim D, Park D, Choi S, Lee S, Sun M, Kim C, Shin HS. Thalamic control of visceral nociception mediated by T-type Ca^{2+} channels. Science 2003; 302:117-9.
113. Dogrul A, Gardell LR, Ossipov MH, Tulunay FC, Lai J, Porreca F. Reversal of experimental neuropathic pain by T-type calcium channel blockers. Pain 2003; 105:159-68.
114. Wen XJ, Xu SY, Chen ZX, Yang CX, Liang H, Li H. The roles of T-type calcium channel in the development of neuropathic pain following chronic compression of rat dorsal root ganglia. Pharmacology 2010; 85:295-300.
115. Todorovic SM, Jevtovic-Todorovic V, Meyenburg A, Mennerick S, Perez-Reyes E, Romano C, et al. Redox modulation of T-type calcium channels in rat peripheral nociceptors. Neuron 2001; 31:75-85.
116. Todorovic SM, Meyenburg A, Jevtovic-Todorovic V. Redox modulation of peripheral T-type Ca^{2+} channels in vivo: alteration of nerve injury-induced thermal hyperalgesia. Pain 2004; 109:328-39.
117. Maeda Y, Aoki Y, Sekiguchi F, Matsunami M, Takahashi T, Nishikawa H, Kawabata A. Hyperalgesia induced by spinal and peripheral hydrogen sulfide: evidence for involvement of $Ca_V3.2$ T-type calcium channels. Pain 2009; 142:127-32.
118. Uchitel OD, Di Guilmi M, Urbano FJ, Gonzalez Inchauspe C. Acute modulation of calcium currents and synaptic transmission by gabapentinoids. Channels (Austin) 2010; 4:490-6.
119. Dworkin RH, O'Connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, et al. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 2007; 132:237-51.
120. Taylor CP. Mechanisms of analgesia by gabapentin and pregabalin—calcium channel $\alpha_2\delta$ [$Ca_V\alpha2$-δ] ligands. Pain 2009; 142:13-6.
121. Bauer CS, Nieto-Rostro M, Rahman W, Tran-Van-Minh A, Ferron L, Douglas L, et al. The increased trafficking of the calcium channel subunit $\alpha_2\delta$-1 to presynaptic terminals in neuropathic pain is inhibited by the $\alpha_2\delta$ ligand pregabalin. J Neurosci 2009; 29:4076-88.
122. Li CY, Song YH, Higuera ES, Luo ZD. Spinal dorsal horn calcium channel $\alpha_2\delta$-1 subunit upregulation contributes to peripheral nerve injury-induced tactile allodynia. J Neurosci 2004; 24:8494-9.

123. Luo ZD, Calcutt NA, Higuera ES, Valder CR, Song YH, Svensson CI, Myers RR. Injury type-specific calcium channel $\alpha_2\delta$-1 subunit upregulation in rat neuropathic pain models correlates with antiallodynic effects of gabapentin. J Pharmacol Exp Ther 2002; 303:1199-205.
124. Newton RA, Bingham S, Case PC, Sanger GJ, Lawson SN. Dorsal root ganglion neurons show increased expression of the calcium channel $\alpha_2\delta$-1 subunit following partial sciatic nerve injury. Brain Res Mol Brain Res 2001; 95:1-8.
125. Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME, Yaksh TL. Upregulation of dorsal root ganglion $\alpha_2\delta$ calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. Journal of Neuroscience 2001; 21:1868-75.
126. Li CY, Zhang XL, Matthews EA, Li KW, Kurwa A, Boroujerdi A, et al. Calcium channel $\alpha_2\delta$1 subunit mediates spinal hyperexcitability in pain modulation. Pain 2006; 125:20-34.
127. Suzuki R, Dickenson AH. Differential pharmacological modulation of the spontaneous stimulus-independent activity in the rat spinal cord following peripheral nerve injury. Exp Neurol 2006; 198:72-80.
128. Mould J, Yasuda T, Schroeder CI, Beedle AM, Doering CJ, Zamponi GW, et al. The $\alpha_2\delta$ auxiliary subunit reduces affinity of omega-conotoxins for recombinant N-type ($Ca_V2.2$) calcium channels. J Biol Chem 2004; 279:34705-14.
129. Hogan QH. Role of decreased sensory neuron membrane calcium currents in the genesis of neuropathic pain. Croat Med J 2007; 48:9-21.
130. Hogan QH, McCallum JB, Sarantopoulos C, Aason M, Mynlieff M, Kwok WM, Bosnjak ZJ. Painful neuropathy decreases membrane calcium current in mammalian primary afferent neurons. Pain 2000; 86:43-53.
131. McCallum JB, Kwok WM, Sapunar D, Fuchs A, Hogan QH. Painful peripheral nerve injury decreases calcium current in axotomized sensory neurons. Anesthesiology 2006; 105:160-8.
132. Nguyen D, Deng P, Matthews EA, Kim DS, Feng G, Dickenson AH, et al. Enhanced pre-synaptic glutamate release in deep-dorsal horn contributes to calcium channel $\alpha_2\delta$-1 protein-mediated spinal sensitization and behavioral hypersensitivity. Mol Pain 2009; 5:6.
133. Hendrich J, Van Minh AT, Heblich F, Nieto-Rostro M, Watschinger K, Striessnig J, et al. Pharmacological disruption of calcium channel trafficking by the $\alpha_2\delta$ ligand gabapentin. Proc Natl Acad Sci USA 2008; 105:3628-33.
134. Bauer CS, Rahman W, Tran-van-Minh A, Lujan R, Dickenson AH, Dolphin AC. The anti-allodynic $\alpha(2)\delta$ ligand pregabalin inhibits the trafficking of the calcium channel $\alpha_2\delta$-1 subunit to presynaptic terminals in vivo. Biochem Soc Trans 2010; 38:525-8.
135. Saheki Y, Bargmann CI. Presynaptic Ca_V2 calcium channel traffic requires CALF-1 and the $\alpha_2\delta$ subunit UNC-36. Nat Neurosci 2009; 12:1257-65.
136. Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, et al. Gabapentin receptor $\alpha_2\delta$-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. Cell 2009; 139:380-92.
137. Perret D, Luo ZD. Targeting voltage-gated calcium channels for neuropathic pain management. Neurotherapeutics 2009; 6:679-92.